JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1996, p. 134–139 Vol. 34, No. 1
0095-1137/96/$04.00+0


# Evaluation of Roche Amplicor PCR Assay for *Mycobacterium tuberculosis*

WENDY L. WOBESER,[1,2] MEL KRAJDEN,[1,2] JOHN CONLY,[1,2] HELEN SIMPSON,[2] BIANCHE YIM,[3] MARIO D'COSTA,[1,3] MILAN FUKSA,[3] CLENCY HIAN-CHEONG,[3] MAX PATTERSON,[1,3] ANNE PHILLIPS,[1,2] ROBERT BANNATYNE,[1,4] ALBERT HADDAD,[5] JAMES L. BRUNTON,[1,2] AND SIGMUND KRAJDEN[1,3]*

*University of Toronto,[1] Departments of Microbiology, The Toronto Hospital,[2] St. Michael's Hospital,[4] St. Joseph's Health Center,[3] and Laboratory Services Branch, Ministry of Health,[5] Toronto, Ontario, Canada*

Received 10 July 1995/Returned for modification 18 August 1995/Accepted 27 October 1995

**The Roche Amplicor *Mycobacterium tuberculosis* PCR test (RMtb-PCR) was compared with mycobacterial culture, with the BACTEC 460 system and inoculation on Lowenstein-Jensen media. Results were interpreted with an adjusted "gold standard" incorporating clinical diagnosis. A total of 1,480 clinical specimens from 1,155 patients, including tissues and fluids, as well as 141 specimens which demonstrated a positive growth index on the BACTEC 460 system were assessed. The sensitivity, specificity, and positive and negative predictive values of RMtb-PCR compared with the adjusted gold standard for clinical specimens were 79, 99, 93, and 98%, respectively. In smear-positive specimens, the sensitivity of RMtb-PCR was 98% versus 53% for smear-negative specimens. When RMtb-PCR was performed two times per week, PCR results were available an average of 21 days before the culture results. For specimens demonstrating a positive growth index on the BACTEC 460 system, RMtb-PCR had a sensitivity and specificity of 98 and 100%, respectively. This study demonstrates the value of a commercial nucleic acid amplification kit for rapid diagnosis of *M. tuberculosis*, particularly in smear-positive specimens or BACTEC culture-positive specimens.**

The importance of tuberculosis as a global public health concern has been emphasized by high incidence rates (particularly in human immunodeficiency virus [HIV]-positive individuals, the homeless, and prisoners) and by recent outbreaks of multidrug-resistant tuberculosis in the United States (3, 7, 13). Factors contributing to the outbreaks have included delays in the diagnosis and implementation of proper infection control measures, as well as delays in the institution of appropriate chemotherapy (5). *Mycobacterium tuberculosis* is easily spread by aerosols, and it is generally recommended that hospitalized patients whose respiratory specimens are smear positive for acid-fast bacilli should be kept in respiratory isolation for 2 weeks until treated. This may be costly, inconvenient, and inappropriate if the isolate is confirmed to be a *Mycobacterium* sp. other than *M. tuberculosis* (MOTT). Conventional diagnosis of *M. tuberculosis* by culture generally takes 3 to 8 weeks. Acid-fast smears lack sensitivity (22) and cannot distinguish *M. tuberculosis* from other mycobacteria. Rapid differentiation of *M. tuberculosis* from other mycobacterial species is therefore of great potential benefit.

The PCR can provide a rapid and specific identification of *M. tuberculosis* complex organisms. Since 1990, more than 25 studies have been published about the detection of *M. tuberculosis* by this method. These studies have employed a number of different genetic elements for amplification (including IS*6110* [9, 12, 15], the 65-kDa antigen [4, 26], and the 38-kDa antigen [14, 24]) and have utilized a number of different DNA extraction, amplification, and detection methodologies. These studies have demonstrated that PCR is a powerful tool for the amplification and detection of mycobacterium-specific nucleic acids. However, concerns about the sensitivity, specificity, and reproducibility of in-house PCR assays which are poorly standardized between centers (21) have slowed the endorsement of this technology by regulatory bodies (6).

Given these concerns, we designed a laboratory-based prospective study with the objective of assessing the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of a commercial nucleic acid amplification kit, the Amplicor *M. tuberculosis* test (RMtb-PCR).

## MATERIALS AND METHODS

**Clinical specimens.** During the 1-year study period, a total of 1,480 specimens from 1,155 patients were processed for mycobacteria. Clinical specimens were submitted to the Mycobacteriology Laboratory of The Toronto Hospital from a consortium of tertiary care and community-based hospitals. Specimens were transported to the laboratory within 2 days of collection and were stored at 4°C until processed. Specimens from contaminated sites (bronchoalveolar lavage [BAL], sputa, tissues, fluids other than cerebrospinal fluid [CSF]) were digested and decontaminated with a final concentration of 2.5% NaOH and *N*-acetyl-L-cysteine and concentrated by centrifugation at 3,500 × *g* for 15 min (19). Tissues were homogenized by either the Stomacher Lab-Blender 80 (Seward Medical UAV House, London, United Kingdom) or by a sterile disposable tissue grinder (Sage Products, Inc., Crystal Lake, Ill.) on the basis of tissue size prior to decontamination and concentration. CSF samples were processed without prior decontamination (see "DNA preparation"). After concentration, a residual volume of 2 to 3 ml remained. One milliliter was aliquoted for PCR testing and stored at 4°C; 0.5 ml was inoculated into a BACTEC 12B bottle, which was incubated at 37°C. The growth indices of the 12B bottles were assessed with a BACTEC 460 instrument (Becton Dickinson, Cockeysville, Md.) twice weekly for the first 2 weeks and weekly thereafter for up to 6 weeks. Another 0.5 ml was inoculated onto a Lowenstein-Jensen (Difco laboratories, Detroit, Mich.) slope, which was incubated in 5% $CO_2$ at 37°C. Slopes were inspected weekly for up to 8 weeks. Smears were stained with auramine-rhodamine and examined under ×400 power with a Leitz epifluorescence microscope (19). Smears were considered positive if at least one fluorescent bacillus was visualized. All positive smears were confirmed by restaining with a modified Kinyoun stain (8) and independently verified by at least two technologists. Specimens from one contributing hospital were divided before decontamination, concentration, and PCR (see Discussion). Culture-positive specimens were identified by the Ontario Provincial Health Laboratory by nucleic acid hybridization testing (Accuprobe; Gen

* Corresponding author. Mailing address: Department of Microbiology, St. Joseph's Health Center, 30 The Queensway, Toronto, Ontario, Canada M6R 1B5. Phone: (416) 530-6268. Fax: (416) 530-6590.

TABLE 1. Species distribution and smear results for cultures growing mycobacteria

| Species | All clinical specimens | | Sputum or BAL | |
|---|---|---|---|---|
| | No. of specimens | No. (%) smear positive | No. of specimens | No. (%) smear positive |
| *M. tuberculosis* | 100 | 56 (56) | 59 | 40 (68) |
| MAC | 57 | 17 (30) | 51 | 15 (29) |
| *M. xenopi* | 43 | 4 (9) | 43 | 4 (9) |
| *M. fortuitum* | 7 | 0 | 7 | 0 |
| *M. chelonae* | 1 | 0 | 1 | 0 |
| *M. gordonae* | 1 | 0 | 1 | 0 |
| *M. kansasii* | 2 | 2 (100) | 2 | 2 (100) |
| *M. szulgae* | 1 | 1 (100) | 1 | 1 (100) |
| Other MOTT | 6 | 1 (17) | 5 | 1 (20) |
| All | 218 | 81 (37)[a] | 170 | 63 (37)[b] |

[a] Fifty-six of 81 specimens (69%) grew *M. tuberculosis.*
[b] Forty of 63 specimens (63%) grew *M. tuberculosis.*

Probe, Inc., San Diego, Calif.) and conventional biochemical testing (19). There were 630 sputa, 445 BALs, 236 biopsies, 90 normally sterile fluids, 52 aspirates, 22 CSF samples, 4 urine samples, and 1 blood sample. In addition, 141 specimens whose growth indices exceeded 10 in BACTEC bottles were processed for RMtb-PCR.

**DNA preparation, amplification, and detection by RMtb-PCR.** PCR was performed according to the manufacturer's instructions with the RMtb-PCR (Roche PCR Diagnostics, Inc., Branchburg, N.J.). DNA preparation was performed in a laminar air flow biosafety hood in an area separated from the specimen processing area. A volume of 100 μl of each specimen (or the vortexed, unconcentrated BACTEC 12B contents) was used for each amplification. Specimens were washed, lysed, and neutralized according to the manufacturer's protocol. CSF specimens were run in duplicate, with one receiving the normal wash treatment and the other not receiving the wash as a precaution against insufficient precipitation of nucleic acids after the wash step. One positive control and two negative controls supplied by Roche were included in every run. Amplification reagents were prepared on the day of utilization. False-positive results due to amplicon contamination were minimized through the use of uracil-*N*-glycosylase (AmpErase). A 35-cycle procedure was used with a GeneAmp PCR system 9600 (Perkin Elmer): 20 s at 94°C, 20 s at 62°C, and 45 s at 72°C. The final elongation step was continued for 5 min at 72°C, and the product was held at 72°C. PCR product detection was performed on the same day as amplification. Amplification products and hybridization buffer were transferred to microwell plates coated with oligonucleotides specific for *M. tuberculosis* complex. After incubation at 37°C for 1.5 h, the plates were washed five times. Avidin-horseradish peroxidase conjugate and substrate were added. Samples were considered positive if the $A_{450}$ was equal to or greater than 0.35.

**Discrepant result analysis.** RMtb-PCR was repeated when possible on specimens which were RMtb-PCR negative and culture positive for *M. tuberculosis*. The medical records of all patients who had positive RMtb-PCR and negative culture results were reviewed by the investigators. Data concerning the clinical presentation, epidemiologic risk factors, past culture results, and previous or current therapy were analyzed. Additional specimens were examined if available. After this analysis, the PCR result was reclassified as appropriate. The combination of culture and clinical data was utilized to generate an adjusted gold standard.

**Statistical methods.** Data were entered into a Epi-Info 6.00 database (Centers for Disease Control and Prevention, Atlanta, Ga.). Continuous variables were compared by Student's *t* test. Sensitivities were compared by McNemar's chi-square test.

## RESULTS

**Species distribution and smear results.** A total of 1,480 specimens were processed from 1,155 patients for both culture and RMtb-PCR. Mycobacterial species were grown from 218 of 1,480 specimens (15%), of which 100 of 218 specimens (46%) grew *M. tuberculosis*. Culture and smear results for the identified mycobacterium species are shown in Table 1. Over half, 56 of 100 (56%), of the specimens which grew *M. tuberculosis* were positive by smear. Among specimens positive for MOTT, 57 of 118 (48%) grew *Mycobacterium avium* complex (MAC) and 43 of 118 (36%) grew *Mycobacterium xenopi* (*M. xenopi* is one of the most common MOTT found in Ontario, Canada [23]). When only respiratory specimens are considered (sputum and BAL), 40 of 59 (68%) of the specimens growing *M. tuberculosis* were positive by smear, as were 15 of 51 (29%) of the MAC isolates and 4 of 43 (9%) of the *M. xenopi* isolates. Of all smear-positive respiratory specimens, 40 of 63 (63%) grew *M. tuberculosis*. A total of eight smear-positive respiratory specimens failed to grow mycobacteria, two of which were positive by RMtb-PCR.

**Comparison of smear, culture, and PCR results.** Table 2 demonstrates the relationship between culture, smear, and RMtb-PCR results excluding BACTEC-cultivated specimens. *M. tuberculosis* was cultivated in 77 specimens which were also positive by RMtb-PCR. *M. tuberculosis* was not cultivated in 1,362 specimens that were also negative by RMtb-PCR. A total of 23 specimens were *M. tuberculosis* positive only by culture, and 18 were positive only by RMtb-PCR. The smear positivity rate was 55 of 77 specimens (71%) in culture-positive specimens which were also RMtb-PCR positive, whereas in specimens which were culture positive and RMtb-PCR negative, the smear positivity rate was only 4%. Details of the discordant results are given in Tables 4 and 5 (see below). The overall sensitivity of RMtb-PCR for specimens which were *M. tuberculosis* culture positive was 77%, whereas the sensitivity for smear was 56%. With McNemar's chi-square test, the difference is significant at $P < 0.01$. The PPVs and NPVs of RMtb-PCR versus culture were 77 and 98%, respectively.

Of all 56 specimens that were smear and culture positive for *M. tuberculosis*, all but 1 (98%) was also positive by RMtb-PCR. Of the 44 specimens negative by smear and positive by culture for *M. tuberculosis*, 22 (50%) were positive by RMtb-PCR. For respiratory specimens, 40 were positive by smear and culture for *M. tuberculosis*, and all were positive by RMtb-PCR. Of the 19 respiratory specimens which were smear negative and culture positive, 9 of 19 were also RMtb-PCR positive (47%). RMtb-PCR was repeated for 6 of the 10 false-negative specimens, but in only one case was the repeated result positive.

The distribution of culture-positive specimens by RMtb-PCR result is given in Table 3. The sensitivities of RMtb-PCR for sputum and BAL were comparable (84 and 81%, respectively). The sensitivity of RMtb-PCR for biopsy specimens was 74% (this difference was not significant). The numbers of *M. tuberculosis* culture-positive aspirates, fluids, and CSFs were too few to draw specific conclusions regarding the comparative performance of RMtb-PCR (Table 3).

TABLE 2. Detection of *M. tuberculosis* by RMtb-PCR versus culture in 1,480 clinical specimens[a]

| Culture result (no. of specimens) | No. of RMtb-PCR-tested specimens (% smear positive) | |
|---|---|---|
| | Positive | Negative |
| Positive for *M. tuberculosis* (100) | 77 (71)[b] | 23[c] (4)[b] |
| Negative for *M. tuberculosis* (1,380) | 18[d] | 1,362[e] |
| Total | 95 | 1,385 |

[a] A total of 1,155 patients provided specimens. Note that BACTEC specimens were excluded.
[b] 71% = 55 of 77 and 4% = 1 of 23. Overall, 56% (56 of 100) of the culture-positive specimens were smear positive.
[c] Details are given in Table 4.
[d] Details are given in Table 5 (includes five MOTT).
[e] Includes 113 MOTT.

TABLE 3. Distribution of specimens which were culture positive for *M. tuberculosis*

| PCR result | No. of samples from: | | | | | |
|---|---|---|---|---|---|---|
| | Sputum | BAL | Aspirate | Biopsy | Fluid | CSF |
| Positive | 36 | 13 | 4 | 20 | 2 | 2 |
| Negative | 7 | 3 | 3 | 7 | 1 | 2 |
| Total | 43 | 16 | 7 | 27 | 3 | 4 |

**Analysis of discordant results with the adjusted gold standard.** Discordance between different detection assays may be related to the assay design or its performance characteristics. For example, DNA amplification techniques may detect nucleic acid from both viable and nonviable organisms. Therefore, patients receiving therapy may have a positive PCR result despite being culture negative. To address this possibility, we incorporated an adjusted gold standard which included clinical and treatment data.

**(i) Culture-positive, RMtb-PCR-negative specimens.** Table 4 gives a detailed analysis of the 24 specimens from 22 patients which were positive by culture for *M. tuberculosis* and negative by RMtb-PCR. The single false-negative RMtb-PCR specimen which was smear positive was on a peritoneal dialysate which had not been concentrated. After concentration of this sample, the RMtb-PCR was positive. The first culture-positive CSF we tested was RMtb-PCR negative after washing as per the manufacturer's directions. Because of concerns that the washing step could have resulted in the loss of amplifiable target, we split subsequent CSFs into washed and unwashed specimens prior to performance of RMtb-PCR. An additional two CSFs were both culture and RMtb-PCR positive—one in both the washed and unwashed specimens and one in only the washed specimen. (One CSF was only positive by RMtb-PCR but was negative on culture. This patient was undergoing treatment [Table 5].) An additional three specimens (one of each biopsy, BACTEC and sputum) were positive on repeat RMtb-PCR, 10 were negative on repeat RMtb-PCR, and 8 were not repeated because of a lack of sufficient specimen. Specimens divided prior to concentration and homogenization (from one hospital site) did not differ from other specimens in the rate of smear, culture, or RMtb-PCR positivity.

**(ii) Culture-negative, RMtb-PCR-positive specimens.** A total of 18 specimens from 16 patients were initially classified as RMtb-PCR false positive with culture as the gold standard (Table 5). However, after review, 11 of 18 specimens (nine patients) were reclassified as true positives, 6 specimens remained as false positives (1 because of laboratory contamination), and 1 specimen was indeterminate. Six of the 16 patients (eight specimens) were undergoing treatment at the time the specimen was obtained (treatment duration ranging from 2 to 130 weeks). The patient who had been on treatment for 130 weeks was HIV positive, known to be noncompliant, and had severe disease, as shown by a chest radiograph. We concluded that all six of these patients were true positives. One patient classified as true positive had been treated for tuberculosis 1 year earlier and had a persistent cavitary lesion. At a 1-year follow-up, without treatment, this patient remained asymptomatic and showed almost complete resolution of the radiological abnormality. Three other patients were started on antituberculous therapy after the RMtb-PCR result, two of three having clinical and histopathological evidence of tuberculosis. One of three patients was from an area endemic for tuberculosis and had bilateral lower lobe pneumonia, which on pathology demonstrated bronchiolitis obliterans and organizing pneumonia. This patient was started on steroids in addition to antituberculous therapy, with a partial clinical response. We classified the two former patients as true positives; the third patient was classified as indeterminate.

Four patients grew *M. xenopi*, all from respiratory specimens. One of the four had severe cavitary disease, multiple positive cultures, and was on therapy; the three remaining had no significant pulmonary disease (one had recently been treated with intravesicular *Mycobacterium bovis* BCG for bladder carcinoma). One other patient with chronic lung disease presumed to be due to MAC and on therapy against MAC was positive by RMtb-PCR on two occasions. One patient (post-liver transplant) with no evidence of tuberculosis was PCR positive, likely as a result of laboratory contamination.

Table 6 summarizes the performance of RMtb-PCR versus culture and the adjusted gold standard. With the adjusted gold standard, the overall sensitivity in clinical specimens was 79% and the specificity was 99%. The PPV was 93%, and the NPV was 98% (Table 6). For smear-positive specimens, the sensitivity was unchanged at 98%, and the sensitivity for smear-negative samples rose to 53%. The sensitivity and specificity of RMtb-PCR with the adjusted gold standard for respiratory specimens were 84% and 99%, respectively.

**BACTEC specimens.** A total of 141 BACTEC specimens with positive growth indices were processed for RMtb-PCR. The average growth index (at the time RMtb-PCR was performed) was 299 (range, 10 to 999). *M. tuberculosis* was cultured from 50 specimens (35%), MAC was cultured from 49 specimens (34%), and *M. xenopi* was cultured from 35 speci-

TABLE 4. Analysis of 24 specimens from 22 patient culture positive for *M. tuberculosis* and negative by RMtb-PCR[a]

| Patient | Specimen | Result by: | | Comment(s) |
|---|---|---|---|---|
| | | Smear | Repeat[b] | |
| 1 | BAL | Negative | NSQ[c] | |
| 2 | Biopsy | Negative | NSQ | Lymph node |
| 3 | CSF | Negative | Positive (wash)[d] | BAL RMtb-PCR positive |
| 4 | BAL | Negative | Negative | |
| 5 | Biopsy | Negative | Negative | Lymph node |
| 6 | Biopsy | Negative | NSQ | Lymph node |
| 7 | Biopsy | Negative | Negative | Pleural |
| 8 | Fluid | Negative | NSQ | Neck abscess |
| 9[e] | Sputum | Negative | Negative | |
| 10 | Biopsy | Negative | Positive | Lymph node |
| 11 | Biopsy | Negative | NSQ | Lymph node |
| 12 | BAL | Negative | Negative | Culture negative twice on the same BAL |
| 13 | BACTEC | Negative | Positive | Lymph node |
| 14 | Sputum | Negative | Positive | |
| 15 | Sputum | Negative | Negative | |
| 16 | Biopsy | Negative | NSQ | Lymph node |
| 17 | CSF | Negative | Negative (wash)[d] | |
| 18 | Fluid | Positive | Positive (concn) | Peritoneal dialysate, unconcentrated specimen was PCR negative |
| 19 | Sputum | Negative | NSQ | |
| 20 | Sputum | Negative | NSQ | |
| 21[e] | Aspirate | Negative | Negative | |
| 22 | Sputum | Positive | Negative | RMtb-PCR run on small volume (less than 100 μl) |

[a] Not tested for the presence of inhibitors.
[b] Result of repeat PCR.
[c] NSQ, insufficient quantity for repeat testing.
[d] CSF specimens were run in duplicate (see Methods).
[e] Patients with two distinct specimens.

TABLE 5. Analysis of 18 specimens from 16 patient cultures negative for *M. tuberculosis* and positive by RMtb-PCR

| Reclassification | Patient | Previous TB[a] | Clinical TB | Specimen | Result by: | | Comment(s) |
|---|---|---|---|---|---|---|---|
| | | | | | Smear | Repeat PCR | |
| Contamination | 1 | No | No | Sputum | Negative | NSQ[b] | No evidence of *M. tuberculosis*, Liver Transplant |
| True positive | 2[c] | Yes | Yes | Sputum | Positive | NSQ | On treatment for 6 wk, also grew *M. xenopi* |
| True positive | 3[c] | Yes | Yes | Sputum | Positive | NSQ | On treatment for 130 wk, noncompliant, HIV positive |
| True positive | 4 | Yes | Yes | Biopsy | Negative | NSQ | On treatment for 20 wk, severe lung disease, new lymph node |
| True positive | 5 | Yes | Yes | Biopsy | Positive | NSQ | On treatment for 26 wk, active pericarditis at surgery |
| True positive | 6 | Yes | Yes | Biopsy | Positive | NSQ | On treatment for 26 wk, persistent lymph node drainage |
| True positive | 7 | Yes | Yes | CSF | Negative | NSQ | On treatment for 2 wk for meningitis |
| True positive | 8 | No | Yes | Aspirate | Positive | NSQ | Treated for TB, apical pneumonia, AFB[d] positive on pathology |
| True positive | 9 | No | Yes | Biopsy | Negative | NSQ | Treated for TB, lymph node AFB positive on pathology |
| True positive | 10 | Yes | No | BAL | Negative | NSQ | Previously treated for *M. tuberculosis* 1 year ago, persistent cavity |
| False positive | 11 | No | No | Sputum | Positive | Yes/negative | Cavitary lung disease, multiple cultures positive for *M. xenopi* only |
| False positive | 12 | No | No | Sputum | Negative | NSQ | Not treated for MTB, lung nodule, 2 specimens positive for *M. xenopi* |
| False positive | 13 | No | No | BAL | Negative | NSQ | Recent treatment with BCG, granuloma on X ray, grew *M. xenopi* |
| False positive | 14 | ? | ? | Sputum | Negative | Yes/negative | Clinical data unavailable, *M. xenopi* |
| False positive | 15 | No | No | Sputum | Positive | Yes/positive | Chronic lung disease with MAC, on steroids, anti-MAC treatment |
| Indeterminate | 16 | No | No | Biopsy | Negative | NSQ | Treated for *M. tuberculosis*, bilateral lower lobe pneumonia, BOOP[e] |

[a] TB, tuberculosis.
[b] NSQ, insufficient quantity of sample for retesting.
[c] Patients 2 and 3 each had separate specimens positive by RMtb-PCR and negative by Culture.
[d] AFB, acid-fast bacillus.
[e] BOOP, Bronchiolitis obliterans and organizing pneumonia.

mens (24%). Two each grew *Mycobacterium chelonae* and *Mycobacterium gordonae*, and one grew *Mycobacterium kansasii*. Two grew MOTT which were not identified to the species level. Of those growing *M. tuberculosis*, 49 of 50 were positive by RMtb-PCR for a sensitivity of 98%. Testing of the one false-negative RMtb-PCR specimen was repeated, and the specimen was found to be positive by RMtb-PCR (Table 4). None of the 91 specimens growing MOTT were positive by RMtb-PCR, for a specificity of 100%. The PPV and NPV for RMtb-PCR as applied to BACTEC were 100 and 99%, respectively.

## DISCUSSION

Our study demonstrates the large-scale application of the commercial RMtb-PCR, a standardized nucleic acid amplification assay, for the rapid diagnosis of tuberculosis. Our results showed that the sensitivity of RMtb-PCR for all non-BACTEC specimens against an adjusted gold standard was 79%. For smear-positive specimens, the sensitivity was 98%, and for smear-negative specimens, the sensitivity was 53%. This was consistent with data reported by others (1, 11, 27,). A survey of state health laboratories using conventional culture revealed that the average time from the receipt of specimens to the reporting of results to a physician is 34 days (16). With BACTEC, this can be shortened to 22 days, and with the addition of nucleic acid probes, this can be shortened to 10 to 14 days. Although we routinely use BACTEC in conjunction with nucleic acid probes, the turnaround time from specimen receipt to result report in our hands was 25 days, compared with 3.7 days for RMtb-PCR when the latter was run twice per week. In a high-volume laboratory, a same-day RMtb-PCR result could be provided.

The number of false-negative results for RMtb-PCR and the need to determine the susceptibility of the isolates illustrate that PCR technology with its current sensitivity must be performed in conjunction with culture. Although 17 of 24 specimens had either been divided prior to concentration and homogenization or were tissue specimens which were divided prior to concentration and homogenization, they did not differ from other specimens in the rate of smear, culture, or RMtb-PCR positivity. Therefore, we do not feel that prior division contributed to the number of false negatives. However, currently all specimens are homogenized prior to division for culture and PCR. We did not test for the presence of inhibitors in our study, which have been reported to occur in 5 to 13% of specimens (4, 20, 25) and could have been responsible for some of the false-negative results.

In the present study, most false-positive specimens (Table 5) were probably due to the presence of nonviable organisms in patients while on therapy. Published experience with PCR supports the concept that a patient can remain PCR positive after cultures become negative. In one study, PCR remained positive 1 to 2 months after cultures became negative (17), and occasionally results can be positive at 6 months after the initiation of therapy (18). Patients that are PCR positive after 6 months of treatment may be at high risk of relapse (17). Beige et al. (2) found a significant number (11 of 24) of positive PCR results in a population of patients infected with *M. tuberculosis* but who were smear and culture negative and had no evidence of clinical disease; we found only one such patient. Only one false-positive specimen was attributed to contamination. In addition, five specimens (four *M. xenopi* and one MAC) may have generated false-positive results. The optical densities of these specimens tended to be lower than those of specimens

TABLE 6. Summary of performance of RMtb-PCR on the basis of culture and adjusted gold standard

| Specimens | Adjusted gold standard [no. (%) of specimens] | | | |
|---|---|---|---|---|
| | Sensitivity | Specificity | PPV | NPV |
| Clinical[a] | 77 (79) | 99 (99) | 81 (93) | 98 (98) |
| Smear positive[a] | 98 (98) | 76 (93) | 86 (97) | 97 (97) |
| Smear negative[a] | 50 (53) | 99 (100) | 71 (81) | 98 (98) |
| Respiratory[a] | 83 (84) | 99 (99) | 82 (90) | 99 (99) |
| BACTEC culture[b] | 98 (98) | 100 (100) | 100 (100) | 99 (99) |

[a] Includes all specimens tested directly by RMtb-PCR (i.e., no BACTEC cultures).
[b] RMtb-PCR results from BACTEC culture (growth indices ≥10).

containing *M. tuberculosis* but were above the assay cutoff. Although these readings may represent a biological false positive, none of 35 BACTEC specimens which grew *M. xenopi* (or the 49 growing MAC) were positive by RMtb-PCR. It is unlikely that these patients were dually infected with *M. tuberculosis*, given that only MOTT were cultured in at least two specimens from all but one patient. We are currently investigating if there is a biological basis for this potential cross-reactivity.

The rapid detection of mycobacteria by smear is hampered by its lack of sensitivity and its inability to differentiate between mycobacterial species. For respiratory specimens, the overall sensitivity of RMtb-PCR was 84% (98 and 56% on smear-positive and smear-negative specimens, respectively). For smear-positive specimens, rapid exclusion of *M. tuberculosis* can save unnecessary respiratory isolation and potential contact tracing costs. Of smear-positive respiratory isolates, 37% grew MOTT (Table 1); this is higher than numbers published by Yajko et al., who grew MOTT from 8% of smear-positive sputa and 29% of smear-positive BAL (28). The differentiation of *M. tuberculosis* from MOTT on smear-positive specimens in a timely fashion also allows for the early institution of appropriate therapy.

This study helps to define the role of PCR assays in the management of patients with suspected tuberculosis. Two areas in which RMtb-PCR performed well and can likely be demonstrated to be cost-effective have been identified. The first area is the early species identification of smear-positive specimens. In this setting, we found, as did D'Amato et al. (11), that a negative RMtb-PCR result virtually excluded tuberculosis. The second setting in which RMtb-PCR may be indicated is in its early application to positive BACTEC specimens. Forbes and Hicks also found PCR to be very sensitive (100%) and specific (99.7%) when applied to BACTEC specimens with a growth index greater than 10 (15). They found the time to identification of *M. tuberculosis* to be 14 days with PCR versus 29 days with nucleic acid probes. In our hands, the RMtb-PCR result was available a mean of 4 days before the results of nucleic acid probe tests. Testing of all BACTEC specimens at the earliest positive growth index could increase this time advantage.

At this time, the Public Health Service recommends that conventional laboratory methods (including BACTEC and nucleic acid probes) be used for the detection of *M. tuberculosis* (6). The appropriate application of a rapid diagnostic tool such as RMtb-PCR can dramatically shorten the time to diagnosis. Given the reliability of RMtb-PCR for smear-positive specimens and specimens positive on BACTEC, we now use RMtb-PCR routinely in these two settings. The use of the RMtb-PCR test on an outsource basis for smear-positive patients is cost-effective in an institution which sees cases of pulmonary MOTT to *M. tuberculosis* at a ratio of smear-positive cases of 1:8 or less by the most conservative isolation cost scenarios for which isolation days may be saved (10). The greatest potential for individual institutional cost savings is in settings with large HIV and immunosuppressed populations, in which both MOTT and *M. tuberculosis* are prevalent. The greatest health care savings are realized when specimens are outsourced from multiple institutions to a single central laboratory facility. To optimize tuberculosis infection control practices and to prevent future outbreaks, early diagnosis of infectious individuals is critical. The availability of highly standardized nucleic acid amplification technologies promises to be an effective tool for attaining this goal.

## ACKNOWLEDGMENTS

We thank the following members of the Toronto Rapid Diagnostics Consortium for providing specimens: A. Born and E. Szentgyorgyi, North York Branson Hospital; M. Karmali, Hospital for Sick Children; D. Low, Mount Sinai Hospital; A. Medline, Northwestern General Hospital; A. Gryfe and A. Perl, Queensway General Hospital; A. Simor, Sunnybrook Health Science Centre; R. Devlin, The Wellesley Hospital; and M. Vearncombe, Women's College Hospital.

## REFERENCES

1. **Beavis, K. G., M. B. Lichty, D. L. Jungkind, and O. Giger.** 1995. Evaluation of Amplicor PCR for direct detection of *Mycobacterium tuberculosis* from sputum specimens. J. Clin. Microbiol. **33:**2582–2586.
2. **Beige, J., J. Lokies, T. Schaberg, U. Finckh, M. Fischer, H. Mauch, H. Lode, B. Köhler, and A. Rolfs.** 1995. Clinical evaluation of a *Mycobacterium tuberculosis* PCR assay. J. Clin. Microbiol. **33:**90–95.
3. **Bloom, B. R.** 1992. Tuberculosis—back to a frightening future. Nature (London) **358:**538–539.
4. **Brisson-Noel, A., C. Aznar, C. Chureau, S. Nguyen, C. Pierre, M. Bartoli, R. Bonete, G. Pialoux, B. Gicquel, and G. Garrigue.** 1991. Diagnosis of tuberculosis by DNA amplification in clinical practice evaluation. Lancet **338:** 364–366.
5. **Centers for Disease Control and Prevention.** 1991. Nosocomial transmission of multidrug-resistant tuberculosis among HIV-infected persons—Florida and New York, 1988–1991. Morbid. Mortal. Weekly Rep. **40:**585–591.
6. **Centers for Disease Control and Prevention.** 1993. Diagnosis of tuberculosis by nucleic acid amplification methods applied to clinical specimens. Morbid. Mortal. Weekly Rep. **42:**686.
7. **Centers for Disease Control and Prevention.** 1993. Tuberculosis morbidity—United States 1992. Morbid. Mortal. Weekly Rep. **42:**696–704.
8. **Chain, K.,** 1995. Clinical microscopy, p. 33–51. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
9. **Clarridge, J. E., III, R. M. Shawar, T. M. Shinnick, and B. B. Plikaytis.** 1993. Large-scale use of polymerase chain reaction for detection of *Mycobacterium tuberculosis* in a routine mycobacteriology laboratory. J. Clin. Microbiol. **31:** 2049–2056.
10. **Conly, J., S. Krajden, M. Krajden, R. Bannatyne, W. Wobeser, and The Toronto Rapid Diagnostics Consortium.** 1995. Conventional vs rapid molecular diagnostic strategies for *M. tuberculosis* (M.tb): a cost-effectiveness analysis (CEA), abstr. D93, p. 83. *In* Program and abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
11. **D'Amato, R. F., A. A. Wallman, L. H. Hochstein, P. M. Colaninno, M. Scardamaglia, E. Ardila, M. Ghouri, K. Kim, R. C. Patel, and A. Miller.** 1995. Rapid diagnosis of pulmonary tuberculosis by using Roche AMPLICOR *Mycobacterium tuberculosis* PCR test. J. Clin. Microbiol. **33:**1832–1834.
12. **Eisenach, K. D., M. D. Sifford, M. D. Cave, J. H. Bates, and J. T. Crawford.** 1991. Detection of *Mycobacterium tuberculosis* in sputum samples using a polymerase chain reaction. Am. Rev. Respir. Dis. **144:**1160–1163.
13. **Ellner, J. E., A. R. Hinman, S. W. Dooley, M. A. Fischl, K. A. Sepkowitz, M. J. Goldberger, T. M. Shinnick, M. D. Iseman, and W. R. Jacobs, Jr.** 1993. Tuberculosis symposium: emerging problems and promise. J. Infect. Dis. **168:**537–571.
14. **Folgueira, L., R. Delgado, E. Palenque, and A. R. Noriega.** 1993. Detection of *Mycobacterium tuberculosis* DNA in clinical samples by using a simple lysis method and polymerase chain reaction. J. Clin. Microbiol. **31:**1019–1021.
15. **Forbes, B. A., and K. E. Hicks.** 1994. Ability of PCR assay to identify *Mycobacterium tuberculosis* in BACTEC 12B vials. J. Clin. Microbiol. **32:** 1725–1728.
16. **Huebner, R. E., R. C. Good, and J. I. Tokars.** 1992. Current practices in mycobacteriology: results of a survey of state public health laboratories. J. Clin. Microbiol. **31:**771–775.
17. **Kennedy, N., S. H. Gillespie, A. O. S. Saruni, G. Kisyombe, R. McNerney, F. I. Ngowi, and S. Wilson.** 1994. Polymerase chain reaction for assessing treatment response in patients with pulmonary tuberculosis. J. Infect. Dis. **170:**713–716.
18. **Levee, G., P. Glaziou, B. Gicquel, and S. Chanteau.** 1994. Follow-up of tuberculosis patients undergoing standard anti-tuberculosis chemotherapy by using a polymerase chain reaction. Res. Microbiol. **145:**5–8.
19. **Nolte, F. S., and B. Metchock.** 1995. Mycobacterium, p. 400–437. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
20. **Nolte, F. S., B. Metchock, J. E. McGowan, Jr., A. Edwards, O. Okwumabua, C. Thurmond, P. S. Mitchell, B. Plikaytis, and T. Shinnick.** 1993. Direct detection of *Mycobacterium tuberculosis* in sputum by polymerase chain reaction and DNA hybridization. J. Clin. Microbiol. **31:**1777–1782.
21. **Noordhoek, G. T., A. H. J. Kolk, G. Bjune, D. Catty, J. W. Dale, P. E. M. Fine, P. Godfrey-Faussett, S.-N. Cho, T. Shinnick, S. B. Svenson, S. Wilson,**

**and J. D. A. van Embden.** 1994. Sensitivity and specificity of PCR for detection of *Mycobacterium tuberculosis*: a blind comparison study among seven laboratories. J. Clin. Microbiol. **32:**277–284.
22. **Pfaller, M. A.** 1994. Application of new technology to the detection, identification, and antimicrobial susceptibility testing of mycobacteria. Am. J. Clin. Pathol. **101:**329–337.
23. **Simor, A. E., I. E. Salit, and H. Vellend.** 1984. The role of *Mycobacterium xenopi* in human disease. Am. Rev. Respir. Dis. **129:**435–438.
24. **Sjöbring, U., M. Mecklenburg, A. B. Andersen, and H. Miörner.** 1990. Polymerase chain reaction for detection of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **28:**2200–2204.
25. **Soini, H., M. Skurnik, K. Liippo, E. Tala, and M. K. Viljanen.** 1992. Detection and identification of mycobacteria by amplification of a segment of the gene coding for the 32-kilodalton protein. J. Clin. Microbiol. **30:**2025–2028.
26. **Telenti, A., F. Marchesi, M. Balz, F. Bally, E. C. Böttger, and T. Bodmer.** 1993. Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. J. Clin. Microbiol. **31:**175–178.
27. **Vuorinen, P., A. Miettinen, R. Vuento, and O. Hällström.** 1995. Direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens by Gen-Probe amplified Mycobacterium Tuberculosis Direct Test and Roche Amplicor Mycobacterium Tuberculosis Test. J. Clin. Microbiol. **33:**1856–1859.
28. **Yajko, D. M., P. S. Nassos, C. A. Sanders, J. J. Madej, and W. K. Hadley.** 1994. High predictive value of the acid-fast smear for *Mycobacterium tuberculosis* despite the high prevalence of *Mycobacterium avium* complex in respiratory specimens. Clin. Infect. Dis. **19:**334–336.